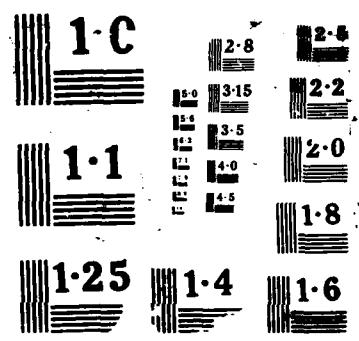


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A physical basis is established whereby external electromagnetic fields can interact with biological membranes. The mechanism discussed, based on the membrane residing in a thermodynamic state near a critical point, can accommodate very low field strengths. Numerical estimates of field strengths required to drive the membrane system close enough to a critical point, assuming it does not initially reside there, are presented.

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## 1.0 CRITICAL PHENOMENA AND ELECTROMAGNETIC EFFECTS IN BIOLOGICAL MEMBRANES

### 1.1 INTRODUCTION

The specific manner in which external electromagnetic fields interact with biological membranes to alter membrane mediated processes such as passive ion transport and ligand binding at the cell surface is not well understood. In other words, a clearly identified physical basis for field/membrane coupling has not been established. A physical basis, however, can be established assuming the membrane can exist in a thermodynamic state that is "near" or "near enough" to a continuous phase transition (Bond and Wyeth, 1986; Bond and Wyeth, 1987). As we have previously demonstrated, a membrane residing in such a state possesses unique dielectric response characteristics. For example the electric susceptibility,  $\chi$ , becomes very large as the critical temperature,  $T_c$ , is approached. That is,

$$\chi \propto \frac{1}{T-T_c} . \quad (1)$$

A large body of both experimental and theoretical evidence now exists that strongly suggests that the gel to liquid crystalline phase transition in lipid bilayer membranes is only weakly first order and is primarily a second order phase transition during which the membrane system resides close to a thermodynamic critical point (Marcelja, 1974; Mitaku et al., 1983).

The unique sensitivity to external perturbations afforded by equation (1) provides a physical basis for the heretofore unexplained coupling to membranes of low intensity electromagnetic fields, e.g. the class of responses generically known as nonthermal responses. In subsequent sections, we show how an external field can "drive" a membrane "close enough" to a critical point such that critical phenomena become important and actually dominate the response of the system to the external field. Also, there may exist *in vivo* membrane systems that initially reside sufficiently close to a critical point without the requirement for being "driven" toward  $T_c$ .

## 1.2 THEORY

In our modeling efforts, we regard the membrane as a two-dimensional hexagonal lattice, each lattice site occupied by a lipid molecule which resides in one of two states.

State A            all trans configuration of acyl chains;

State B            any "excited" state (rotational isomers of acyl chains: kinks and/or jogs).

Head groups are initially assumed to have a given orientation which may remain fixed even in the presence of an external field.

Employing lattice statistics via the Lenz-Ising model we can immediately write

$$T_c = \frac{0.6068}{8k} qW \quad (2)$$

where:     q = coordination number;

W = interaction potential energy between nearest neighbor;

k = Boltzmann's constant.

Clearly if W can be altered,  $T_c$  follows suit.

Can an external field, then, alter W?

The answer is definitely yes, but before we describe how this can be accomplished, we need to examine the nature of W.

Contributions to W can be conveniently subdivided into:

1. head group - head group interactions ( $W_{h-h}$ )

and

2. chain - chain interactions ( $W_{c-c}$ ).

For a lipid component such as dipalmitoylphosphatidyl choline (DPPC), the head group interaction is predominantly a dipole-dipole interaction. Thus,

$$W_{h-h} = \frac{p_i \cdot p_j - 3(N \cdot p_i)(N \cdot p_j)}{4\pi\kappa\epsilon_0 |r_i - r_j|^3} \quad (3)$$

where  $p_i$  = dipole moment vector,  
 $N$  = unit vector between  $p_j$  and  $p_i$ ,  
 $\kappa$  = dielectric constant,  
 $\epsilon_0$  = permittivity of free space,  
 $r_i$  = position vector of  $i^{\text{th}}$  dipole.

The  $p_i$  are characteristically of the order of 25 debye in magnitude.

Now since changes in the chain (hydrocarbon tail) configuration are the primary feature of the main lipid bilayer gel/liquid crystalline phase change, the nature of the chain-chain interactions is very important. Principal contributions to the chain-chain interaction are the van der Waals' attraction and the excluded volume repulsion. The acyl chains are nonpolar with restricted orientation. The van der Waals interaction is therefore primarily by way of the dispersion contribution to the overall interaction potential.

For identical nonpolar molecules, as in the chain-chain interaction, the dispersion interaction is governed approximately by

$$W_{c-c} = \frac{-3}{4} \frac{I}{(4\pi\kappa\epsilon_0)^2} \frac{\alpha^2}{R^6} \quad (4)$$

where  $R$  = intermolecular separation,  
 $\alpha$  = molecular polarizability,  
 $I$  = first ionization potential.

Changes in  $W_{h-h}$  can occur through a reorientation of the  $p_i$  initiated by an external field,  $E$ , and/or an induced dipole contribution. Changes in

$W_{c-c}$  can occur through: (a) nonlinear polarization (hyperpolarization), i.e.  $\alpha = \alpha(E)$ , (b) induction of oriented dipoles within the chain, and (c) excitation of chain-chain vibration modes, i.e. enhanced oscillations of the chains about their equilibrium position would alter  $W_{c-c}$ .

### 1.3 NUMERICAL ESTIMATES

Consider a DPPC bilayer with the head groups arranged in the plane of the bilayer and the chains in the fully extended configuration. The total interaction potential energy for two adjacent molecules can be written as

$$W = W_{h-h} + W_{c-c} . \quad (5)$$

From equation (2)

$$\Delta W = (3.0 \times 10^{-23} \frac{J}{K}) \Delta T_c \quad (6)$$

where we have assumed a hexagonal lattice ( $q = 6$ ). Mitaku et al. have experimentally shown that the "distance" between  $T_c$  and  $T_t$  is 0.6K. (Here  $T_t$  is the phase transition temperature for DPPC.)

Thus, from equation (6)  $\Delta W = 1.8 \times 10^{-23} J$  in order for  $T_c$  to coincide with  $T_t$ , assuming that  $T_t$  did not change appreciably with the change in  $\Delta W$  (see Bond and Wyeth, 1986).

Now according to equation (3)

$$W_{h-h} = \frac{-p^2}{2\pi\kappa\epsilon_0 R^3} . \quad (7)$$

Taking  $p = 25$  debye,  $\kappa = 10$ , and  $R = 5 \times 10^{-10} m$ , we obtain  $W_{h-h} = -9.8 \times 10^{-20} J$ . Experimentally it has been determined that  $W_{c-c} = 2 \times 10^{-19} J$  (based on a measured value of 1.84 kcal/mole  $CH_2$ ).

Therefore we have

$$\frac{\Delta W}{W_{h-h}} = 1.8 \times 10^{-4} , \quad (8)$$

and

$$\frac{\Delta W}{W_{c-c}} = 9 \times 10^{-5} . \quad (9)$$

What is the magnitude of E needed to bring about these changes in W.  
From equation (7)

$$\frac{\Delta W_{h-h}}{W_{h-h}} = \frac{-2\Delta p}{p} \quad (10)$$

Now  $\Delta p = \alpha \Delta E$  where  $\Delta E = E_{\text{external}}$ .

Taking  $(\alpha/4\pi\epsilon_0) = 1.8 \times 10^{-30} \text{ m}^3$  ( $\alpha = 2 \times 10^{-40} \frac{\text{m}^2\text{C}}{\text{V}}$ ),

$$\frac{\Delta p}{p} = 9 \times 10^{-5}$$

yields  $\Delta p = 2.25 \times 10^{-3}$  debye (or  $7.51 \times 10^{-33}$  C-m). Therefore

$$\Delta E = E_{\text{ext}} = \frac{\Delta p}{\alpha} = 3.75 \times 10^7 \text{ V/m} . \quad (11)$$

From equation (4),

$$\frac{\Delta W_{c-c}}{W_{c-c}} = - \frac{2\Delta\alpha}{\alpha} \text{ for R constant.}$$

Hyperpolarizability effects can be estimated by using an effective susceptibility:

$$x_{\text{eff}} = x + 2Ed \quad (12)$$

where E is the applied field and d is the nonlinear coefficient. For  $x \approx 2 \times 10^{-11} \text{ c/v-m}$  for alkane chains, we can estimate  $d \sim 4 \times 10^{-23} \text{ c/v}^2$ .

Taking the approximation

$$\frac{\Delta\alpha}{\alpha} \approx \frac{\Delta\chi_{\text{eff}}}{\chi_{\text{eff}}} = (\frac{x}{2d} + E)^{-1} \Delta E$$

$x/2d \sim 3 \times 10^{11}$  V/m >> reasonable  $E_{\text{ext}}$ .

Thus for  $|\frac{\Delta W_{c-c}}{W_{c-c}}| = 9 \times 10^{-5}$  (from equation 4)

$$\frac{\Delta\alpha}{\alpha} \approx 4.5 \times 10^{-5}$$

and

$$\Delta E \approx 1.5 \times 10^7$$
 v/m.

If oriented dipoles are induced (case b above),

$\Delta W_{c-c} = 1.8 \times 10^{-23}$  J,  $r = 5 \times 10^{-10}$  m implies  $p \approx 5 \times 10^{-31}$  c-m = 0.15 debye for  $p = \alpha E$  with  $\alpha \sim 2 \times 10^{-40}$  m<sup>2</sup>-c/v then  $E \approx 2.5 \times 10^9$  v/m.

#### 1.4 SUMMARY AND CONCLUSIONS

Mechanisms exist whereby an external EM field can change the interaction between biomembrane molecules. We have identified and discussed some (but not all) of these mechanisms.

All mechanisms discussed above require field strengths comparable to or greater than endogenous field strengths "seen" by in vivo membrane systems.

The excitation of a normal mode of vibration of the bilayer appears to be the most likely candidate for a driving mechanism. This could account for the frequency specificities observed experimentally.

Experiments designed to test our critical phenomena hypothesis are needed.

### 1.5 SUGGESTED EXPERIMENTS

Experiments to test our overall critical phenomena hypothesis in biological membranes and their lipid bilayer analogues could be readily designed and performed. Standard techniques using light scattering (critical opalescence), ultrasound relaxation measurements (relaxation time and relaxation strength), and membrane permeability anomalies near  $T_c$  could all be employed.

### 1.6 REFERENCES

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## 2.0 ELECTROPORATION AND ELECTROFUSION IN CELL BIOLOGY

### 2.1 BOOK

A book, Electroporation and Electrofusion in Cell Biology, is currently being edited by E. Neumann, A. Sowers and C. Jordan. This multi-authored book will fulfill a vital need that exists for an authoritative review on the use of electric fields to evoke cell fusion, electroporative gene uptake, or injection of biologically significant objects into cells. Plenum Press has agreed to publish the book and a contract has been signed.

### 2.2 HISTORY

The Office of Naval Research submitted a proposal to the American Association for the Advancement of Science (AAAS) to conduct a Symposium at the 1986 Annual Meeting in Philadelphia on "Electrofusion and

Electroporation of Cells and Protoplasts." The AAAS not only accepted the proposal for the Symposium, but allowed a somewhat smaller session of related "Contributed" papers to follow the Symposium. Simultaneously, in Bielefeld, Germany a workshop was being conducted on "Mechanisms of Membrane Processes, Electric Gene Transfer and Cell Fusion." As those responsible for both these sessions became aware of each others efforts, the idea for a book, Electroporation and Electrofusion in Cell Biology, emerged. Some, but not all, of the participants in both sessions were invited to submit an "original" appraisal of their research interests related to the book topic. Others were invited.

### 2.3 STATUS

The Dedication, Preface and twenty-six Chapters have been received and virtually all have gone through an initial editing phase. No chapters are outstanding. One of the chapters has been withdrawn because it fell below an acceptable standard.

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